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**VEGETATION STRUCTURE OF A COSTA RICAN FOREST
FRAGMENT AND THE GENETIC STRUCTURE OF THE
INHABITANT *ALOUATTA PALLIATA* POPULATION**

**A thesis submitted to
Regis College
The Honors Program
in partial fulfillment of the requirements
for Graduation with Honors**

by

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May 2016

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I. Introduction

In the following pages, I present my argument on the dangers of industrial agriculture, as exemplified by deforestation for the growing of tropical fruit that has occurred throughout the history of Costa Rica. Principally, I contend that the system of large-scale food growth and consumption by urban populations in industrial countries indicates a disconnection between this human population and the environment that produces its sustenance. In response to this issue, I turn to scientific investigation of the world as a means of informing the participants of industrialized living about the consequences of land use. Then, I present my findings on how deforestation at a site in northeastern Costa Rica, which has been fragmented for tropical fruit cultivation and cattle ranching, relates to the structure of the vegetation in the forest fragment and the genetic structure of the inhabitant population of mantled howler monkey, *Alouatta palliata*. My findings that forest edge exhibits different vegetation structures than the interior and that the *A. palliata* population indicates signs of inbreeding imply a tangible relationship between fragmentation and the variables I observe.

My work on this project started at the beginning of my junior year when I began working with Dr. Franco and Dr. Schreier on their collaborative study on the role of kinship and social development in white-faced capuchins, *Cebus capucinus*, and mantled howler monkeys, *Alouatta palliata*, at the La Suerte Biological Research Station (LSBRS) in Costa Rica. To study kinship, I was given the opportunity to help analyze the

genetic characteristics of these monkeys, and I soon became interested in the work. Drs. Schreier and Franco also informed me of the deforestation in the area surrounding LSBRS, which seemed to hold some importance given our initial finding, which suggested genetic structure might be influenced by forest fragmentation. I became invested in the laboratory techniques and the data we were generating, so I seized the opportunity to develop my own project and hypotheses about the genetic structure of the howler monkeys living in the forests around the LSBRS. To collect the data I needed in order to test my hypotheses, I traveled to Costa Rica in the summer of 2015 and learned how to study the structure of the forest, the behavior of the monkeys, and collect fecal samples for DNA analysis.

I decided to approach the genetic analysis of the *A. palliata* population from the perspective of edge effects, which can broadly be defined as the behavioral or physical responses of an organism in relation to the edge, or boundary between habitats (Lidicker, 1999). My focus narrowed to the observation of how vegetation and the genetic structure of the howler monkey population related to the forest-agriculture edge. I characterized tree size, density, and canopy cover. I also measured the overall heterozygosity of *A. palliata* at LSBRS as well as the genetic differentiation among subpopulations sampled at the edge and interior of the forest fragment.

Out of the many firsts I experienced while in Costa Rica, two of the most intriguing were seeing pineapple and banana plants. Along with nearly all of the other life forms in the region, these were completely foreign to me, despite the fact that I had eaten many banana and pineapple fruits throughout my life. The company names and logos of Dole

and Chiquita on the sides of plantation buildings were also familiar sights in a strange environment. It became clear to me that many areas where forests once were had been converted for the purpose of tropical fruit cultivation. The contrast between the alien landscape's strange plant life and the familiar brand names and products struck me in a strange and uneasy way.

After spending three weeks at LSBRS, the exotic plants and animals began to seem less foreign. On our class' journey out of the rainforest and back to life in the Western world, the juxtaposition of the commercial fruit farms and the towering rainforests took on a new meaning to me. I began to notice that the connection between the trees and monkeys at LSBRS and the farming operations outside the forest walls went far beyond my hypotheses of edge effects. The monkeys I had been studying, though perhaps in peril due to low genetic diversity, seemed utterly at home in their environment. On this journey, I gained awareness of the system of food cultivation and consumption I partake in. Industrial nations such as the United States and European countries consume some fruits from tropical regions such as Costa Rica without physical contact between the consumers and the land and people who produce what they eat. I couldn't help but think how indirect the method of human food consumption was when compared to that of the howlers roaming the trees looking for leaves and fruits. Non-human life is restricted by the immediate environment it occupies for the food that is available in a given time and place.

One possible way to bridge the gap between industrialized society and the environment that provides its food is the process of science. The scientific method,

involving observations, hypotheses, methods, results, and interpretations, is a fitting tool to evaluate the state of the natural world in relationship to human activity. What follows is a historical perspective on how human beings have interacted with one particular environment—forests in Costa Rica—along with empirical evidence of its ecological structure in hopes of evaluating how the entire industrialized human world perceives and relates to the rest of the natural community of life.

In Chapter II, I discuss the means of human survival in industrialized nations. Food is obtained from farming regions that exist outside of the urban centers they feed. This creates a distant relationship between humans, one of Earth's many species, and the environment that sustains us. In Chapter III, I explore science's role of informing industrialized civilization. Science is a unique tool that belongs to our species, and its importance to the industrialized nations is now more prevalent than ever. Industrialization might leave its mark through deforestation, but applied scientific research can help inform this type of human activity to ameliorate the disconnection I identify. In Chapter IV, I report on deforestation's impacts on the structure of plant life at LSBRS at one of its two fragments, known as the Large Forest. I conclude that there is a measurable relationship between distance of the forest to the edge—created by commercial agriculture—and tree size, canopy structure, and ground cover characteristics. In Chapter V, I report my findings on the genetic structure of *A. palliata* also inhabiting the Large Forest fragment at LSBRS. My prediction that vegetation characteristics at the edge have promoted gene flow to the interior creating a more heterozygous interior subpopulation than edge subpopulation is not supported. However, my results do indicate that the

population as a whole exhibits signs of inbreeding depression, possibly due to the fragmented nature of the habitat and its restrictions on immigration.

Other than the information I present below, writing this thesis has been a truly transformative experience. In order to fully solidify my thoughts and observations, I had to go further than academic research and scientific investigation. Indeed, the viewpoints expressed in the following pages are as much a result of my own meditation, reflection, and interpersonal interactions as they are the results of scholastic work. My answers to the questions I bring up are unfinished, but I hope and fully expect to continue working on my solutions throughout my existence.

II. Agriculture in Costa Rica and the trend toward commercialization

Modern industrial civilization thrives in densely populated cities fed by rural farmland. In the United States, for example, eighty percent of the population lives in urban areas (The World Bank, 2016). In other words, two hundred and fifty million people live in U.S. cities. The year-round availability of food at supermarkets anywhere in the nation is arguably one of this civilization's greatest accomplishments, but it deserves some detailed evaluation. To explore industrialized civilization's relationship with the natural world, I elaborate on the historical, economic, and social factors that have molded agriculture from a process done on a small scale into a large commercial enterprise. This is exemplified by deforestation to make room for tropical fruit plantations and cattle pastures, which has occurred in Costa Rica—the nation that serves as my thesis' point of reference. Industrialized human life, as it exists today, removes human beings from the natural world, apparent in how food is produced and consumed in nations like the U.S. Yet, as I outline in the next chapter, the universal human capacity to empirically know the environment presents itself as an imperative to the industrial way of life in order to avoid total environmental alienation. Finally, I present two examples of applied scientific research within the context of deforestation for commercial agriculture.

The present chapter identifies some underlying motivations of industrialized society's disconnect from the environment in order to contextualize my scientific analyses. While travelling to collect biological data in a northeastern Costa Rican forest at the La Suerte Biological Research Station (LSBRS), I observed the after-effects of deforestation in the form of banana and pineapple farms directly adjacent to towering, dense trees. In recent decades, tropical forests around the world have been cleared at significant rates for a variety of reasons. Costa Rica experienced an intensive period of forest clearing that ranked among the fastest in the world during the 1960's and 70's (Rosero-Bixby & Palloni, 1996), creating numerous forest fragments that still house life (which I explore in Chapters IV and V). Deforestation occurred near LSBRS during this time for cattle farming, pineapple cultivation, and other agricultural purposes (Garber et al., 2010). In Latin America, reasons for deforestation include subsistence and commercial agriculture, wood extraction for domestic use, and clearing space for transportation routes (Geist & Lambin, 2002). Changes in a land's agricultural productivity, including deforestation, are intrinsically linked to growth in human population size and, perhaps more importantly, density (Boserup, 1965). Human deforestation occurs under various circumstances as the result of different social systems, but it more often than not serves as one step in the production of food. That is, the human effort to obtain food for survival is apparent in land use.

Civilized life centers around the benefits of agriculture. Farming first appeared around 10,000 years ago in the Fertile Crescent and many early cultures began cultivating plants around the same time (Balter, 2013). Domesticating plants and animals

revolutionized how humans live. Surpluses coincided with the formation of large villages soon connected by trade networks (Balter, 2007), and these dense communities began to develop economically and culturally (Boserup, 1965). Farming techniques became more intensive, increasing agricultural efficiency in order to feed large urban populations (Boserup, 1965). This is the foundation of industrial agriculture, which can be defined as “any of various non-manufacturing processes, especially ones relating to plant or animal resources, carried out on a large, commercial scale” (“industrial” Oxford English Dictionary, 2016). To support dense urban centers, farming grew to an industrial scale, visible in the banana-growing operations of Costa Rica as well as the large-scale farming practices of the U.S. and other nations. Over time, farming has transitioned from a technique done for survival into a process capable of supporting massive populations occupied with specialized tasks other than subsistence.

In pre-Columbian Central America, the primary occupation was farming, and this way of life is still evident in small villages throughout the region (Woodward, 1985). Farming traditionally took place on small plots where peasants lived a subsistence lifestyle (Woodward, 1985). Before the Spanish conquest of the area, dense populations and evolving farming techniques did permit specialization and formation of civilizations, like the Maya (Woodward, 1985). In the centuries prior to Spanish colonization of Costa Rica, indigenous populations fluctuated but generally increased, relying on maize cultivation (Clement & Horn, 2001). Over time, the intensity of maize production increased likely due to growing populations (Hoopes, 1996), an observation consistent with Boserup’s (1965) hypothesis that population growth pressures farming development

and intensification. Civilization did exist previous to the conquest, forests were noticeably altered, and the people traded goods (Denevan, 1992). Although, indigenous life differed from modern industrial civilization because the agricultural products that supported the pre-Columbian population came from the same environment the people inhabited. Even trade was regional, such as cacao distribution in Central American (Bergman, 1969).

Beginning in the 16th century, Costa Rica abruptly transitioned from local and regional contact to global interaction with the arrival of the Spanish Empire. At the time, European society was shifting toward industrialization and urbanization, with a decreasing agricultural employment (Allen, 2000), resembling modern Western Europe and the U.S. So, European countries came to the New World during a time of transition when cultural and economic benefits of dense and permanent settlements were being realized (Boserup, 1965). The disparity between the urbanized European model and the New World populations that generally practiced subsistence provides a useful backdrop for analyzing the tensions between the industrialized, human-made world and the environmentally bounded natural world that pre-Columbian Americans and all non-human organisms occupy.

Juan de Cavallón, the first Spanish conquistador to lead expeditions to present-day Costa Rica, wrote a letter to the Spanish Crown that stated, “this province offers great wealth, with fertile soil...it has oranges and lemons, and we believe it will produce other fruits” (Palmer & Molina, 2006, p. 15). Spain emphasized production and export of tropical produce not available in the mother country to bolster the nation’s wealth

(Woodward, 1985). It makes sense that de Cavallón stressed Costa Rica's potential for fruit production. Early in the Spanish colonial period of Central America, it was clear that exports would guide the region's economic future (Woodward, 1985). Spain sought to profit materially off the land, which juxtaposed the indigenous subsistence lifestyle. Pursuing food as a source of material wealth makes land valuable for reasons other than its capacity to sustain life on the basic level. In de Cavallón's words and in Spain's intentions, land is a commodity. This mindset supports a separation between humankind and the environment, as the latter is thought to serve the former.

After many Latin American countries gained independence from Spain in the 19th century, foreign investors began to take advantage of the region's valuable natural resources. The United Fruit Company (UFCO) notably emerged during this period as a leading producer of bananas. In hopes of giving their newly independent country an economic advantage in the increasingly globalized world economy, Costa Rican leaders encouraged the construction of railroads to aid in transport, communication, and commerce (Woodward, 1985). Lacking the capital to undertake this project domestically, the Costa Rican government hired the U.S. railroad builder Henry Meiggs, in return for land grants along the route (Woodward, 1985). Meiggs' nephew, Minor Cooper Keith, soon took over the project and eventually began clearing the forests along the railroad lines to plant bananas, founding the Tropical Trading and Transport Company that would join with the Boston Fruit Company in 1899 to create UFCO (Woodward, 1985).

The banana export industry soon "became in effect a giant foreign-controlled plantation... it needed large acreages, which it usually got by concessions from the

government” (Woodward, 1985, p. 181). Banana industry paralleled land use in the colonial period because land was sought not for survival purposes but for business interests. Furthermore, foreigners often held these interests; a large portion of Costa Rica’s crop yield fed and benefitted those who lived elsewhere—in the U.S. and Europe. Today, bananas are the most-consumed fresh fruit in the United States (United States Department of Agriculture Economic Research Service, 2015), despite the fact that bananas cannot grow in this country. It is thus possible for a population to live in urban centers and consume foods from a distant and foreign environment. Large cities require large farms to sustain them, which in the tropical lowlands of Costa Rica, need to be deforested before they are cultivated. What emerges is a situation in which non-farming populations have an influence on the land that grows its food. A fundamental connection between consumers and the industrial farms that support them cannot be avoided: people will always need food grown somewhere to survive. When a massive urban population demands fruits grown in areas that must first be deforested, however, the land that provides nourishment is fundamentally altered, changing the dynamics of the human-environment connection.

During the 1960’s and 70’s, deforestation rates in Costa Rica rose to some of the highest in the world (Rosero-Bixby & Palloni, 1996), which is attributed to the growth of a different agricultural product: beef. Cattle differ from tropical fruits in that they can be raised in the U.S. Williams (1986) argues that the period between World War II and the 1970’s saw some of the most prosperity in post-industrial Western civilization, changing what these citizens ate, most noticeable in the increase of red meat (Williams, 1986). Just

as railroad and plantation construction required deforestation in Costa Rica, land was granted to ranchers to clear for pasture use, allowing them to raise cattle for sale on local and global markets, especially to U.S. fast food hamburger restaurants (Williams, 1986). U.S. markets demanded lean grass-fed beef, and Costa Rica's tropical climate permitted year-round grazing, creating a cheaper final product. One recurrent theme for deforestation in Costa Rica is to maintain the affordability and availability of primary goods in places like the U.S. where people are willing to consume them.

Consumption of goods grown in Costa Rica connects consumers to the environment that produced the food. But, life in an urban environment obscures this link with grocery stores that sell tropical fruit and fast food restaurants that serve low-priced beef. Conceiving of food as a commodity influences the estrangement of industrial metropolis-dwellers from the land that feeds us. But, it is also possible that the capability of the Earth to produce basic nourishment is overlooked. The mural, "Allegory of Coffee and Bananas" by Aleardo Villa (1897), which hangs in the National Theater of Costa Rica, celebrates workers harvesting coffee and bananas and bringing the goods to European and U.S. ships (Figure 1). According to Williams (1986, p. 120), "absent from the painting are corn, beans and rice... land is for export." The mural does capture a respect for the land's ability to produce goods, something that impressed de Cavallón as well (Palmer & Molina, 2006). But, it focuses on the export economy, rather than on the food that feed the vast majority of the people living on the land. It depicts a departure from admiring the Earth's fertility for the sake of human life itself, instead focusing on the wealth the land can produce for sale to foreign nations.



Figure 1: *Alegoría del café y el banano*, or “Allegory of Coffee and Bananas”, mural in the National Theatre of Costa Rica (Villa, 1897).

Costa Rica’s history as an export economy indicates a human trend toward reliance on land to produce material wealth, but it could also be reflective of the usefulness of economic and social development in terms of human survival. Among the successful class of export farmers in Central America, “great honor and admiration are still bestowed on those forebears who took the initiative to carve from inhospitable territory” the farms and estates that yielded wealth through export (Williams, 1986, p. 119).

Developing dense Costa Rican rainforest into a viable export economy is certainly a feat for the human species, and a respectable undertaking from certain perspectives. Perhaps the emphasis on export and infrastructure expansion is evidence of the human adaptation to survive in the difficult forested environment. Today, thanks largely to the forebears and state leaders who helped develop Costa Rica, it is currently considered one of the most advanced welfare states in the region (Wilson, 1998). Industrial agriculture permits humans to live in cities and to develop services that increase the standard of living,

defined as levels of safety, costs of goods and services, housing, and comfort (Fontinelle, 2008). With commercial banana production, individuals in developed nations can purchase food at a low price, saving time to focus on tasks other than subsisting. Deforestation's contribution to the availability and affordability of healthy food options is a potentially desirable goal for the human species in terms of its own biological and evolutionary success. But, this only considers life from the standpoint of *Homo sapiens*.

Ultimately, an increasingly urbanized civilization that relies on industrial farming falls short of directly integrating human life into the natural world. Examples of deforestation in Costa Rica illustrate the destructive potential stemming from treating the land as an object to be used rather than a resource for people to directly interact with to produce life. Urban living in its current form, while beneficial to the human species in terms of developing a higher standard of living for at least those who can afford it, loses contact with the environment that supports it. Although, to maintain the current standard of development in industrialized nations, not every person can concentrate on farming. I do not support a reversion of human society to pre-agricultural times in which the progress and technologies of civilized life are denounced, such as anarcho-primitivism maintains (Moore, 2009). Instead, I contend that a real connection with the natural world is valuable and that it is made more difficult within the current human set-up exemplified by industrial farming, especially the farming and selling of foods like bananas, fruit that isn't necessary for human survival in countries like the U.S.

The subsistence practices of pre-Columbian Central Americans were mentioned previously, but considering some foundations of subsistence informs why deforestation

for industrial farming harms humanity's standing in the natural world. Veronika Bennholdt-Thomsen and Maria Mies (1999) discuss a subsistence-based alternative to industrially supported civilization. As they correctly point out, not everyone, even in Western populations, lives a life that depends on monetary income, or even has the opportunity to. They emphasize the importance of "work that is expended in the creation, re-creation and maintenance of immediate life" (Bennholdt-Thomsen & Mies, 1999, p. 20). This refers to subsistence living, but it applies, in some manner, to all human work. Their (Bennholdt-Thomsen & Mies, 1999) thesis is that human consumption should only include what is absolutely necessary, in order to directly maintain and create life. In many ways, the end purpose of deforestation is also to continue human life. It permits the growth of crops and cattle that feed growing populations, and the commercial farming industry that takes place of the forests pays workers, albeit minimally, to continue living. But, Bennholdt-Thomsen and Mies (1999) point out that a lifestyle supported by industrial structures assumes that the money-based market, rather than the Earth itself, sustains human life in an indirect manner. I add that one reason such an assumption is easy to make is the disconnection between life in a city and the lands where its nourishment grows. From my conclusions in this chapter in addition to the standpoints on the values of a subsistence life, it follows that the population sustained by industrial processes must embrace some means of knowing and understanding the natural world that supports it.

Human civilization, as it exists in countries of the West and where industrialization supports the maintenance of human life, tends away from a direct connection with the

environment. This seems to be a result of efforts to efficiently sustain larger populations, the desire to make life in urban centers more affordable and comfortable, as well as the supposition that land serves the purpose of profit making. When compared to humans living with the bare minimum, or those who just subsist, a new way of perceiving how industrial societies conduct the basic processes of life appears. The condition and health of the environment is more important, or at least more immediate, to people who rely on and interact with it daily for food. Humans who subsistence farm, in addition to all other forms of non-human life, are necessarily in harmony with the natural world, there is no other option. A city cut-off from the land that produces the foods for its survival has the opportunity to forget what the purpose of land is. It becomes acceptable to clear vast swaths of forest for the production of a delicate luxury like bananas at an affordable price. In the next chapter, I point to scientific investigation as one means of connecting industrialized civilization with the natural world that supports it by means of creating a better understanding of how processes of deforestation act to sustain and support many people.

III. Science informing industrialized life

The repercussions of an increasingly industrialized world can be evaluated from an objective, scientific perspective. One result of global, large-scale agricultural trade of bananas and cattle grown in Costa Rica is deforestation. Although progressing from an agrarian society to industrialization and large-scale efficiency may be a desirable and beneficial way for humans to live, I argue in the previous chapter that it enables human separation from the environment by alienating consumers from the land that produces their nourishment. Here, I propose the strengths of science—both as a defined methodology and as a general search for truth through observation—in improving the relationship between industrialized civilization and the natural world. Science, insofar as it is defined as “study that deals with a connected body of demonstrated truths or with observed facts” (“science” Oxford English Dictionary, 2016), quantifies the natural world into understandable and useful units. By studying edge effects in the plant and howler monkey populations in the anthropogenically-modified forest at LSBRS, I assess the relationship between deforestation and non-human life. The consumer populations of industrial, non-agrarian nations are linked to landscapes where their food grows. But, this connection is distorted when the primary purpose of food production is no longer the maintenance and creation of immediate life. To strengthen its association with the

environment that sustains it, industrialized society can utilize the human tool of scientific, empirical observation.

One of science's primary roles is to quantify and make sense of the natural world. Jacob Bronowski (1985, p. 274), a biologist, argues that scientific knowledge "has as its aim the truth about the nature of things, and so it imposes an obligation to be true to the nature of things—inanimate as well as living nature." This description of science applies to the entire human population. Indeed, in Bronowski's (1985) argument, he implies that aiming at knowing truth is an exercise familiar to all people, across cultures. I present examples of systematically contributing to scientific knowledge in the succeeding two chapters. This is one form of aiming to know the truth about the ecological impacts of tropical deforestation. Although narrow in scope and methods, evaluating the world with the scientific method does contribute to the "discovery of new truth in [the discipline's] own domain" ("science" Oxford English Dictionary, 2016). Systematic science contributes to human perception of the world, through the gathering and interpreting of facts.

Though reasons for seeking the truth about the natural world may vary, in general, "science is inseparable from the collecting and ordering of experiences, gained in the struggle for existence" (Bohr, 1945, p. 1). In other words, accumulating knowledge about how the natural world works is a human solution to meet the challenges of life and to survive. It is a fundamental and defining characteristic of the human species (Bronowski, 1985). Given my position that industrialized civilization is capable of large-scale environmental destruction deriving from the separation of humankind from the natural

world, it follows that the human capability to know the world through experience emerges as a starting point for mitigating this issue. However, this only makes sense with the assumption that in order for *Homo sapiens* to survive as a biological species we must feel the need to come into direct and meaningful contact with the environment.

Charles Darwin, through the process of detailed observation of the natural world, deduced that due to the production of more offspring than are able to survive given limited resources, a struggle for existence ensues in which those organisms possessing profitable traits “under the complex and sometimes varying conditions of life, will have a better chance of surviving” and reproduce more (1872, p. 17). The theory of evolution by natural selection states that environmental pressure, or the conditions of life, plays an important role in the development of species. From a scientific evaluation of the natural world, Darwin theorized that the environment plays a large role in influencing which traits are adaptive and passed along and which are not. Most importantly, what the theory of natural selection brings to the current argument is the point that all forms of life are, to some extent, products of the environments they inhabit. I do not suppose people on Earth would argue against the fact that they are suited to breathe the air of the atmosphere and walk upright despite gravity’s pull. But, the concept that humans are suited to exist in the immediate environment is challenged by the current global connectivity that permits people to survive on the fruits of another land’s and another people’s labor. Which, as explored in Chapter II, has the potential to cause extreme habitat loss for non-human organisms that have no choice but to succumb to the varying conditions of life that humankind imposes.

Undertaking a scientific study on the Costa Rican forests that have fallen to contribute to the nourishment of the industrial world I occupy serves as an example of how science can bring a civilization back into contact with the environment that sustains it. Subsistence living has much to teach the industrialized and commercialized population. One perspective posits that those living at the subsistence level are not hindered from arriving “at a realistic assessment of the present situation and acting accordingly” (Bennholdt-Thomsen & Mies, 1999, p. 7). In the argument of their work *The Subsistence Perspective* (Bennholdt-Thomsen & Mies, 1999), this passage points to a subsistence lifestyle’s immediate connection with the Earth. That is, a person who subsists must know the environment in order to see it produce food; this is a form of science or truth seeking. I do not contend that populations supported by industrialized agriculture are not able to arrive at a realistic assessment of the environment they occupy, but I do agree that it is more difficult for such a population to comprehend the link between tropical deforestation and the nice yellow banana on the supermarket shelf. Here is where science, especially methodological investigation of the impacts of deforestation, is able to provide industrialized societies with a realistic assessment of the present situation.

Examining the characteristics of a tropical forest’s vegetation and the genetic structure of the inhabitant monkey population may initially appear unrelated to the health of the human species. Given the social and economic factors leading to the deforestation of these forests, however, it is apparent that consuming goods grown from the deforested land has linked urban, industrial nations to the tropical soil that grows its food. From this

perspective, the health of many people benefits at the expense of lost and altered habitat for many more non-human organisms. Although destruction of life to sustain another is natural and not inherently bad, what is worrisome is the industrial disconnection between destroying and consuming. To standardize and commercialize the goods that nourish human life is dangerous insofar as it permits the destruction of an ecosystem without a consideration of the impacts on the non-human lives that actually inhabit that landscape. In the following two chapters, I offer examples of how the human quest to know some form of truth about the world can better inform how the human population perceives the Earth that feeds us.

IV. Vegetation structure of a fragmented forest at the La Suerte Biological Research Station, Costa Rica

Introduction

Costa Rica's expanding agricultural export businesses have created a demand for cleared land. Deforestation is a response to the complex interactions between population growth, economic forces, and an increasingly globalized economy (Geist & Lambin, 2002). In the process, lands once covered by tropical forests are converted into valuable farmland. Deforestation for the industrial fruit plantations of Dole and Chiquita connects fruit consumers with many non-human organisms. The need to understand civilization's impact on the natural world exists today stronger than ever. I undertook a project to better this understanding, and it focuses on the vegetation and mantled howler monkey, *Alouatta palliata*, inhabiting a forest fragment cleared for banana, pineapple, and cattle industry in northeastern Costa Rica at the La Suerte Biological Research Station (LSBRS). In order to quantify this impact, I report on vegetation structure at the forest edge and interior in this chapter and the relationship between edge and interior *A. palliata* subpopulations and genetic diversity in the next chapter.

Costa Rica is home to a great diversity of life. The National Biodiversity Institute of Costa Rica predicts that over 500,000 species inhabit the country, and has one of the greatest levels of biodiversity in the world (INBio, 2016). In this study, I observe the

relationship between life, in the form of vegetation, and one result of forest clearing, the formation of an ecological edge. Although deforestation occurs throughout the world, it has been especially prevalent in Costa Rica during the last 50 years. Since World War II, 50% of Costa Rica's territory has been cleared of its primary forests, although the trend toward forest clearing has shown signs of slowing and even reversal in recent years (Rosero-Bixby & Palloni, 1996). In the 1960s and '70s, the rates of forest clearing peaked, and this is when the forest around LSBRS in northeastern Costa Rica experienced the most destruction (Rosero-Bixby & Palloni, 1996; Garber et al., 2010). Clearing of tropical rainforests has implications including the loss of biodiversity, especially in terms of plant diversity (Benitez-Malvido & Martinez-Ramos, 2003). Additionally, it can change landscape characteristics in various ways including habitat destruction, isolation of forest fragments, and the formation of an edge between forested and cleared areas (Skole & Tucker, 1993). Humankind's embrace of agriculture initiated extensive landscape alterations, changing native vegetation across the world (Saunders et al., 1991). Changing land use permits civilization to feed itself, but a close observation of how far deforestation's effects reach is needed to fully understand the issue.

Edge effects are changes in the structures and characteristics of ecosystems near the boundary between two types of habitats (Murcia, 1995; Lidicker, 1999). When forest fragmentation results from agricultural land use, the fragments are surrounded by vegetation that differs from forested land—for example at LSBRS, the land around the Large Forest is primarily grassland. The low biomass and structural complexity of grassland creates changes in microclimate near the edge as more solar radiation can reach

the ground than continuous, light-absorbing canopy (Murcia, 1995). The forest's edges are also exposed to more wind penetration than forest at the interior (Laurance et al., 1998). Edge effects can impact both flora and fauna (Wilcove et al., 1986). The forest edge is a boundary to migration, as both plants and animals move around in their environment (Wilcove et al., 1986). Edges are subject to different seed dispersal patterns, which can lead to species composition at the edge with shade intolerant plants becoming more dominant than shade tolerant plants (Wilcove et al., 1986). Animal species may be less successful at forest edges due to factors including predation (Wilcove et al., 1986). Indeed, the creation of a habitat edge often induces many different ecological phenomena (Chen et al., 1992). This study specifically focuses on the floral characteristics in relation to the forest edge.

Edge effects on vegetation, more specifically, include changes in plant size, understory ground cover, and canopy structure (Broadbent et al., 2008). I define canopy as the upper layer of overlapping mature tree crowns. In tropical forests, canopy and sub-canopy cover decreases near a fragment edge (Williams-Linera, 1990; Laurance, 1991). Forest fragments are also characterized by greater canopy openness—a measurement for the amount of light that reaches the forest floor—as distance to the edge decreases (Gehlhausen et al., 2000). Degree of canopy cover can be measured directly by observing the amount of openness between tree crowns (Gehlhausen et al., 2000), or indirectly by quantifying ground cover. Past findings report a strong positive correlation between canopy openness and the proportion of herbaceous ground cover (Anderson et al., 1969) likely due to the positive correlations between canopy openness and sun light/energy that

reaches the tropical forest floor (Smith et al., 1992). Tree size is also subjected to edge effects in fragments. Trees with a diameter at breast height (DBH) of less than 5 cm were found in higher density near the edges of tropical rainforest fragments in Panama (Williams-Linera, 1990), and saplings, trees with smaller trunks than mature trees, are more prevalent the edge of mesophytic forests (composed of plants that grow in both dry and wet environments); Gehlhausen et al., 2000). Tree density, in both young and old forest edges in a Mexican rainforest, tends to be higher near the forest edge, though this trend appears to become less pronounced as the forest fragment ages (Williams-Linera et al., 1998). LSBRS has had fragmented forests for at least 15 years, so I predict that tree density will not differ between the edge and interior.

Studying the vegetation characteristics of fragmented forests is important for understanding how flora responds to deforestation. These trees also house various animal species. Thus, also at risk is the mantled howler monkey, *Alouatta palliata*, a primarily folivorous arboreal New World primate (Milton & May, 1976). *A. palliata* can survive in isolated forest patches (e.g., Estrada, 1982), as they have at LSBRS forest. However, their long-term fitness in fragments also depends on processes like inbreeding depression, food shortages, and increased hunting accessibility; thus, there is a need to understand how a changing habitat influences this species' behavioral ecology and fitness (Bicca-Marques, 2003). *A. palliata* prefer trees with a large DBH to those with a smaller DBH (Estrada & Coates-Estrada, 1996). Furthermore, DBH correlates positively with tropical fruit abundance (Chapman et al., 1992), which is an indicator of high quality habitat for *A. palliata* (Arroyo-Rodriguez & Mandujano, 2006), as they will eat fruit when available.

DBH is directly proportional to canopy depth, or the 3-dimensional space created by the tree crown (Kanazawa et al., 1990). Canopy depth is a good indicator of *A. palliata* habitat quality (Glander, 1979). Thus, the variables I measure—DBH, canopy cover, ground cover, and depth of canopy that trees occupy—characterize the vegetation structure for its own sake, as well as for evaluating the habitat of *A. palliata* as it relates to distance from the forest edge.

In order to determine whether or not there is a relationship between fragmentation caused by deforestation and the structure of a forest fragment's vegetation, I designed a study to quantify the characteristics of the forest edge versus the forest interior. I hypothesize that the edge forest will be characterized by trees with smaller DBH, have sparser canopy cover, and have increased herbaceous ground cover when compared to interior forest; and that tree density will not differ when comparing edge to interior, and forest level of the trees will be similar in both fragments. In the next chapter, my vegetation studies help to inform my hypotheses on *A. palliata*'s response to deforestation for commercial purposes.

Methods

Study Site

The study site is along the Río La Suerte in northeastern Costa Rica (83°46'15"W, 10°26'30"N). The area consists of lowland Caribbean rainforest and agricultural plantations. The forest near LSBRS is characterized by primary and secondary forest and comprises two fragments, the Large and Small Forests, which are a combined 150 ha (Garber et al., 2010). I studied the structure of the Large Forest from May through June

2015. The non-forest environment surrounding the Large Forest varies in composition and is not continuous. A large portion of the eastern border is composed of pastureland. The northern edge borders a marshland. The western edge of the forest borders an old plantation that is no longer in use and is now characterized by grassland. The southern edge is the least distinct, but is bordered by areas cleared for housing and other human use (Figure 2). Plantations in the surrounding area serve the Dole, Del Monte and Chiquita fruit companies. I define the edge as beginning at the border between tropical rainforest and any surrounding environmental structure that is not tropical rainforest. Any forest within 50 m of the boundary is edge and any forest greater than 50 m from the boundary is interior.



Figure 2: Satellite image of study site at LSBRS. Fragment in red box is the Large Forest, bordered by agricultural plantations on the east and west. Small Forest outlined in yellow. (Google, 2016, image date: 4/9/2013).

Vegetation Sampling

I sampled vegetation using line transects conducted in a similar manner to Arroyo-Rodriguez and Mandujano (2006) and Gehlhausen et al. (2000). I arranged 10 50 m x 5 m transects with the transect started at the forest edge (edge transects), and 10 50 m x 5 m transects positioned at least 50 m from the edge (interior transects). Half of all edge and interior transects were positioned north-to-south, and the other half were positioned east-to-west, in order to control for different amounts of sun exposure (adapted from Gehlhausen et al., 2000). Transect locations were ultimately chosen at random from a number of prospective locations selected for ease of access in order to minimize vegetation disturbance.

For both edge and interior transects, I recorded all trees with a DBH ≥ 10 cm that had at least 50% of the trunk volume within the transect boundaries (Arroyo-Rodriguez & Mandujano, 2006). For each measured tree, I classified it as residing in the canopy layer if its crown was continuous with the neighboring tree crowns. I classified trees with crowns noticeably below the canopy layer as understory, and crowns noticeably above the canopy layer as emergent. I recorded canopy cover and ground cover at every 1 m interval along transects. I quantified canopy cover by assigning each interval to one of 4 cover categories of cover density (1 = 0-25%, 2 = 25-50%, 3 = 50-75%, 4 = 75-100%). I determined the cover category by looking vertically through a cardboard tube (diameter ≈ 4 cm) and estimating the percentage of the tube's area covered by leaves at that interval, assigning it to one of the four cover classes. I quantified ground cover by assigning each meter to a pre-determined category (bare ground, fallen leaves, herbaceous plants/ferns,

fallen woody substrate). When multiple ground cover types characterized a single interval, I categorized the interval using the cover that was closest to the transect tape (for example, if fallen woody substrate or leaves were beneath the leaves of a herbaceous plant, I classified the interval under the “herbaceous plants/ferns” category).

Data Analysis

I calculated mean DBH as the average of all trees found in each of the transect types (i.e., separate averages for all edge and all interior transects). I calculated tree density as the number of trees summed from all transects of each type divided by the total area covered by all of the transects (each individual transect covered an area of 250 m^2 , so each transect type included $2,500 \text{ m}^2$ of sampled land). For canopy cover and ground cover, I averaged the total number of transect intervals classified as each category over the total number of intervals sampled (each individual transect included 50 intervals, and each transect type included a total of 500 intervals sampled). I present percentages of the total intervals sampled as each category for canopy cover and ground cover measurements. I averaged the number of trees recorded in the understory and canopy over the total number of trees sampled for each transect type.

I compared mean DBH, mean tree density, percentages of canopy cover categories, percentages of ground cover categories, and tree depth between edge and interior transects. For statistical analysis, I used a student's t-test and defined significance as a p-value of less than 0.05. I calculated error bars using 95% confidence intervals.

Results

Data gathered from transects indicate that trees at the forest interior have a larger trunk size as well as denser canopy cover than trees at the forest edge. Between edge and interior transects, the trees were equally as likely to be categorized as residing in the understory and the canopy. In both edge and interior forest more trees were found in the understory layer than in the canopy. Overall, tree density was equal between edge and interior forests. Finally, the ground characteristics differed between edge and interior forests, with more fallen leaves and woody substrate found on interior forest floors and more herbaceous plant cover characterizing edge forest floors.

I recorded a mean tree DBH of 29.0 cm along edge transects and a mean tree DBH of 38.4 cm along interior transects of (Figure 3). This difference was statistically significant ($p = 0.049$).

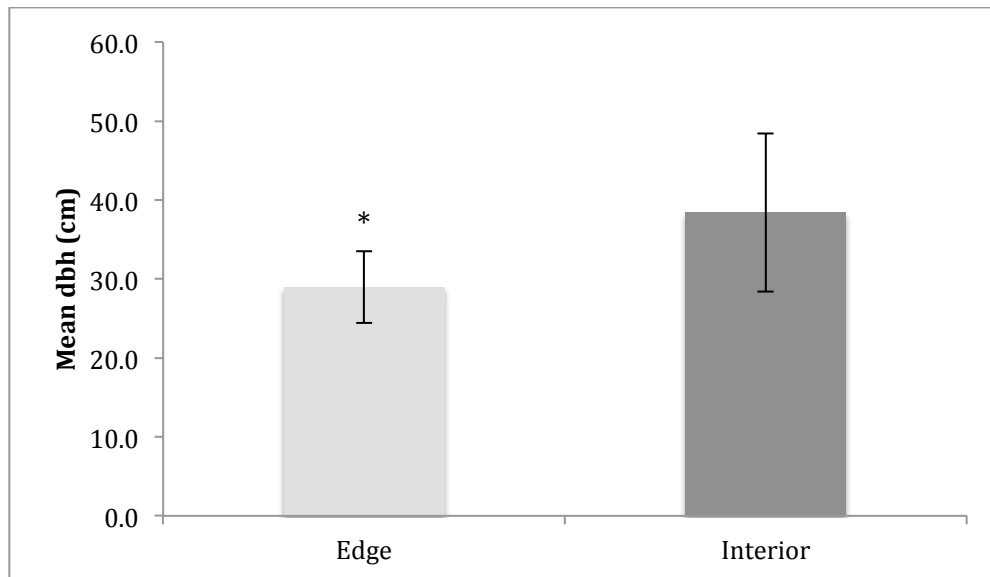


Figure 3: Mean DBH for trees located within 2.5 m of edge and interior transects. Interior trees had a significantly greater DBH than edge trees. Significant differences between edge and interior transects: * $p < 0.05$. Error bars calculated with 95% confidence intervals.

I recorded a tree density of 0.04 trees/m² along edge transects and a tree density of 0.04 trees/m² along interior transects ($p = 0.429$; Figure 4)

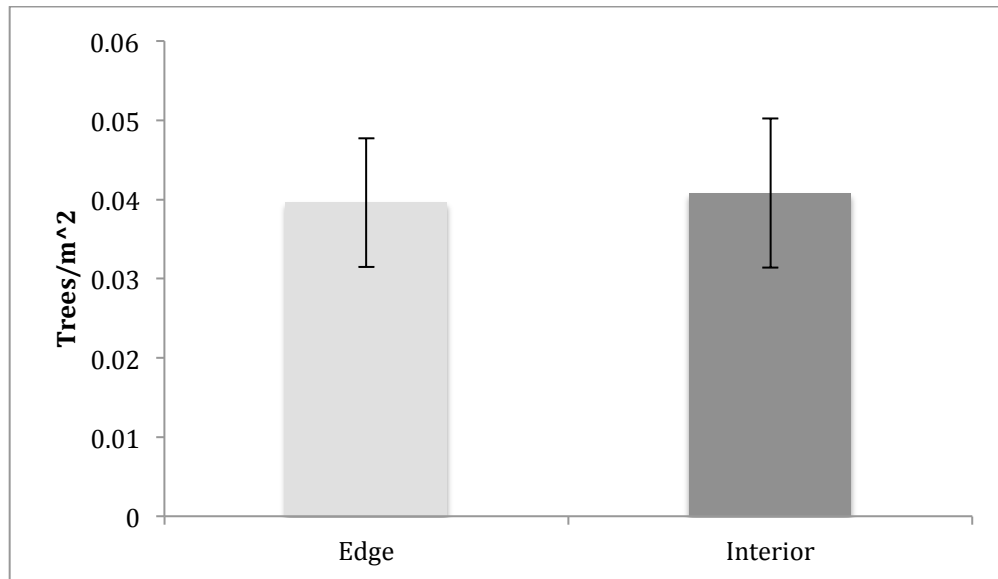


Figure 4: Tree density, reported as trees per square meter, for edge and interior transects. No significant difference was found between tree density at the edge and at the interior. Error bars calculated with 95% confidence intervals.

I found an average of 6.6 and 6.5 trees per each edge versus interior transect, respectively, in the understory. I found an average of 3.3 and 3.7 trees per each edge versus interior transect, respectively, in the canopy (Figure 5). There was no statistically significant difference in the number of trees in each level of the forest between edge and interior transects ($p = 0.471$ for understory, $p = 0.383$ for canopy). However, when considering trees measured in edge and interior transects together, a mean of 6.6 were found in the understory and a mean of 3.5 were found in the canopy. Thus, for both edge and interior transects, there were significantly more trees in the understory than in the canopy ($p = 0.0011$).

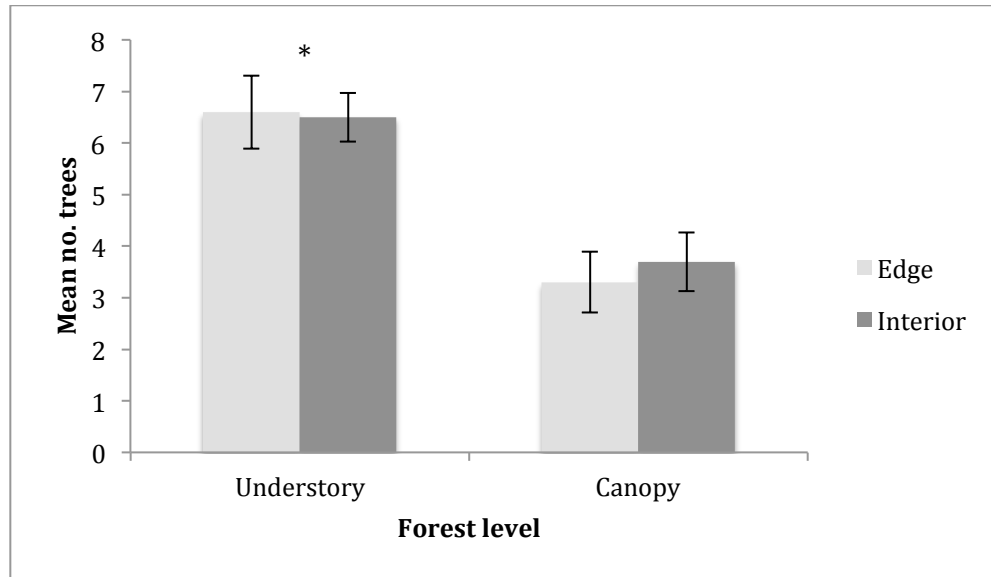


Figure 5: Mean number of measured trees that resided in the understory and canopy out of the total number of edge trees and interior trees. No significant difference between edge and interior was found. Mean number of trees in the understory was significantly different from the mean number of trees in the canopy. Significant differences between understory and canopy trees: * $p < 0.05$. Error bars calculated with 95% confidence intervals.

Ground cover was significantly different between edge and interior transects for the fallen woody substrate, fallen leaves, and plant categories ($p = 0.0022, 0.016, 5.8 \times 10^{-5}$, respectively). Fallen woody substrate and fallen leaves were greater for interior transects, while herbaceous plant cover was greatest for edge transects (Figure 6). The mean number of intervals characterized by bare ground was not statistically significant ($p = 0.37$).

Edge transects had 23.4% of intervals fall in the 0-25% canopy cover range and 17.4% in the 25-50% range compared to interior transects' 3.2% and 6%, respectively (Figure 7). Interior transects had more intervals with an estimated canopy cover of 76-100%, at 68%, compared to 36% of edge transects (Figure 6). Of the four canopy cover categories, significant differences were found between edge and interior transects for the

0-25%, 25-50%, and 75-100% ranges ($p = 0.013$, 0.0011 , 0.0013 , respectively). There was no significant difference between edge and interior transects for the 50-75% canopy cover category ($p = 0.39$).

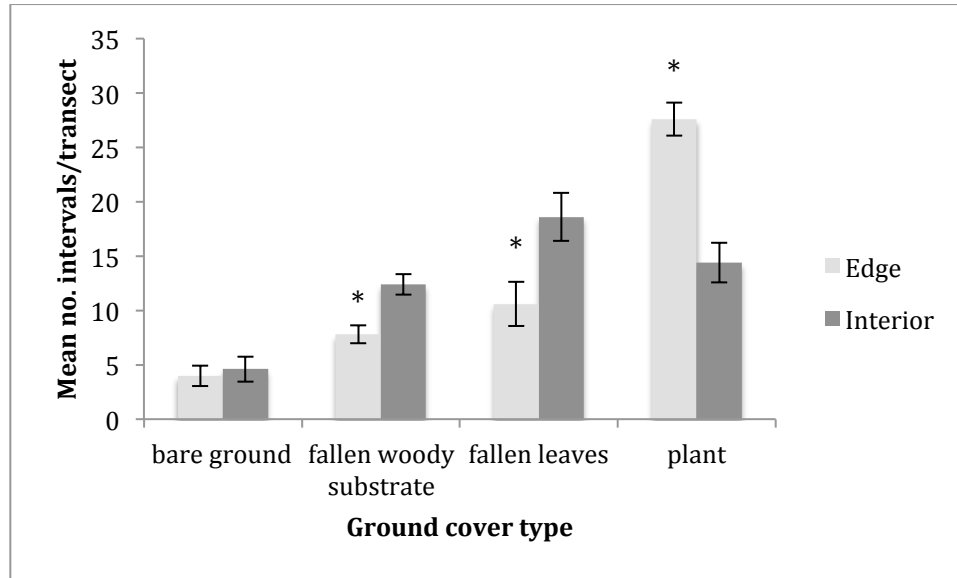


Figure 6: Mean number of intervals of edge and interior transects with bare ground, fallen woody substrate, fallen leaves and herbaceous plant (plant) as the predominant ground cover. Edge transect intervals were more often covered by herbaceous plants, while a greater number of interior intervals, on average, were covered by leaves and fallen woody substrate. Significant differences between edge and interior transects: * $p < 0.05$. Error bars calculated with 95% confidence intervals.

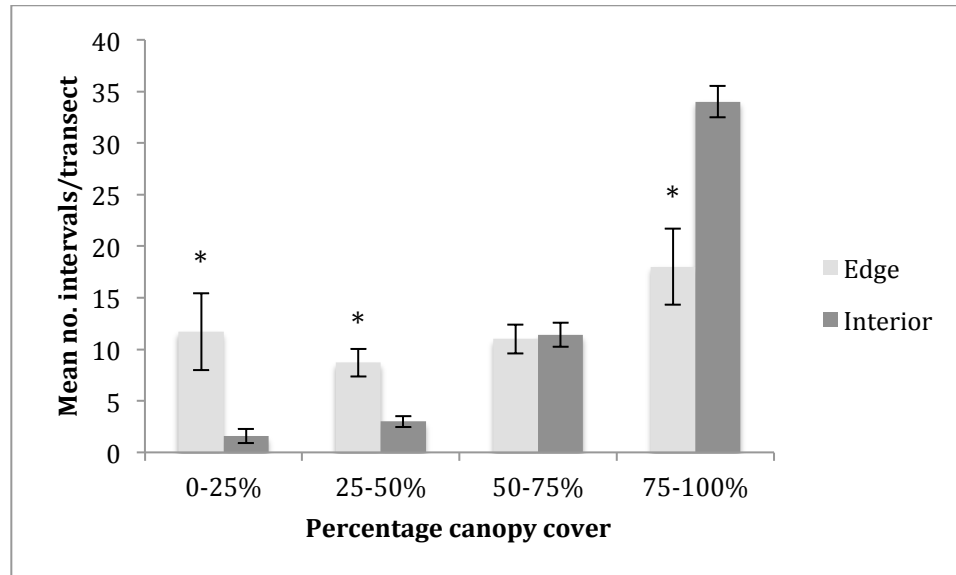


Figure 7: Mean number of intervals of edge and interior transects with 0-25%, 26-50%, 51-75% and 76-100% canopy cover. Edge transects had more intervals fall in the two lowest canopy cover classes while interior transects had a larger number of intervals with an estimated canopy cover of 76-100%. Significant differences between edge and interior transects: * $p < 0.05$. Error bars calculated with 95% confidence intervals.

Discussion

My findings support the hypothesis that the Large Forest at LSBRS has measurable vegetation edge effects. The hypothesis that the edge of the Large Forest at LSBRS will have less dense canopy cover and trees with smaller DBH than the interior forest is supported by my data. These data agree with previous findings that more saplings and immature trees, as indicated by smaller DBH measurements, exist near the forest edge (Williams-Linera, 1990; Gelhausen et al., 2000). Canopy cover is also reported as less dense near the edges of a tropical forest in Panama (Williams-Linera, 1990), which agrees with my findings. My hypothesis of sparser canopy cover in edge forest is further supported by my finding that a greater proportion of edge transects had herbaceous plants covering the ground than interior transects. Past findings have reported a strong positive correlation between canopy openness and the proportion of herbaceous ground cover

(Anderson et al., 1969) along with positive correlations between canopy openness and sun light/energy that reaches the tropical forest floor (Smith et al., 1992). The finding that tree density is equal in both the edge and interior supports my hypothesis that the edge and interior would have similar tree densities, potentially due to the fragment's age (Williams-Linera, 1990). Because some of the forest edges at the Large Forest were originally created at least 15 years ago, the lack of difference in mean tree density may be explained by the older age of the forest edges. One study reports that tree density in a Mexican forest fragment has a less-pronounced relationship with distance from the forest boundary as edges become older (Williams-Linera et al., 1998).

This study is limited by the accuracy of my measurements, particularly with respect to the canopy cover data, as it was difficult to avoid subjectivity while estimating canopy cover. I aimed to arrange transects in random locations, but this proved difficult due to the density of the forest and locations were often altered based on accessibility in the field. Further studies should include classification of tree species in edge and interior forests, as this would provide a more comprehensive picture of the extent of the edge effect beyond tree structure.

These findings have implications for *A. palliata* as forest characteristics including DBH and canopy cover are important when determining habitat quality for arboreal primates. Trees with small DBH are less frequently used by *A. palliata* than trees with large DBH (Estrada & Coates-Estrada, 1996), and fragments characterized by trees with large DBH values are more likely to be occupied by *A. palliata* (Arroyo-Rodriguez et al., 2007). DBH correlates positively with tropical fruit abundance (Chapman et al., 1992),

which is an indicator of high quality habitat for *A. palliata* (Arroyo-Rodriguez & Mandujano, 2006). So tree trunk size measurements indicate the forest interior consists of high-quality trees for *A. palliata* habitation and feeding. DBH positively correlates to canopy depth (Kanazawa et al., 1990), and large canopy depth, along with a continuous canopy (i.e., tree crowns in close proximity to each other), is an indicator of good *A. palliata* habitat (Glander, 1979; Estrada & Coates-Estrada, 1996). Tree trunk size combined with canopy cover measurements indicates that the interior forest at LSBRS comprises high-quality *A. palliata* habitat. Altogether, the structural vegetation data reported in this study show that interior forest comprises higher quality *A. palliata* habitat than edge forest. Deforestation for the commercial agricultural production of bananas and pineapples is shown here to relate to the forest structure insofar as deforestation creates a distinct forest edge. In the next chapter, I combine the findings from this chapter into an analysis of the *A. palliata* population's genetic diversity for evidence of the vegetation edge effects' impacts on their behavior.

V. Using polymorphic microsatellite markers to determine genetic structure of the *Alouatta palliata* population at the La Suerte Biological Research Station, Costa Rica

Introduction

My findings reported in the previous chapter indicate a relationship between the forest edge—created in part by farming tropical fruit—and the structure of the La Suerte Biological Research Station (LSBRS) Large Forest’s vegetation. Most notably, I report that trees at the forest edge have a smaller mean diameter at breast height (DBH) and sparser canopy cover than trees at the forest interior. To further evaluate the repercussions of anthropogenic land change on the species at LSBRS, I conducted a genetic survey of the *Alouatta palliata* population living in the Large Forest fragment. Here, I investigate deforestation’s impacts on a non-human primate species by integrating forest structure data into my hypotheses about how *A. palliata*’s genetic structure relates to the spatial distribution of habitat quality. Both scientific studies presented in this thesis illustrate the interconnectedness of ecosystems, both human and non-human. When a forest is destroyed and fragmented, the dynamics of the biome change beyond the formation of a boundary. There is a connection between commercial farming that feeds cities and the natural world, but it may be initially inconspicuous. Studies like the one presented here help to solidify the connection between environmentally alienating

activities such as industrial farming and life in the natural world, providing information on the impacts of deforestation.

Habitat edges are the boundaries between different habitat-types (Lidicker, 1999). Deforestation creates forest fragments, which are characterized by edges. In Costa Rica and around LSBRS, tropical fruit plantations and cattle pastures form ecological boundaries when adjacent to forest. As continuous forests are increasingly cleared and fragmented, the proportion of forest in contact with the edge increases relative to the amount of interior forest sheltered from the edge. The previous chapter contains a more detailed discussion of edge effects as they impact both flora and fauna. Animal behavior, more specifically, is impacted by habitat edges, though exactly how varies among specific situations (Laurance et al., 1997; Lidicker, 1999). A complete understanding of the effects edges produce depends on elucidating the mechanisms that dictate animals' edge-related behaviors (Lidicker, 1999). In this study, I focus on the dispersal of genetic information in relation to the forest edge, thus, the dispersal behavior of *A. palliata*, especially in fragmented forests, is of particular interest.

Arboreal primate species living in tropical forest fragments can be edge-tolerant with high population densities near the edge, edge-intolerant with high population densities in the forest interior, and omnipresent meaning population densities are nearly equal between edge and interior environments (Lehman et al., 2006). Primate responses to edges are still largely unclear, but Lehman et al. (2006) report that biotic edge effects on food quality is correlated with arboreal primate distribution in a species of lemur. Edge-related behavior of *A. palliata* has yet to be investigated. *A. palliata* is a versatile species,

known to survive in fragments—one study reports group sizes (around 9 individuals) and home ranges in a fragmented population comparable to populations inhabiting continuous forests (Estrada, 1982). This is due, in part, to *A. palliata*'s adaptable diet of leaves (Cristobal-Azkarate & Arroyo-Rodriguez, 2007) and fruit when abundant (Estrada et al., 1999). *A. palliata* persistence in fragments could also be attributed to their ability to occupy relatively small home ranges (Estrada & Coates-Estrada, 1996) that can be between 7-17 ha, though this depends on forest size (Estrada, 1982). From a cursory observation, the presence of *A. palliata* inhabiting a fragment can be interpreted as a sign of good environmental health. One of the goals of my thesis, however, is to highlight the usefulness of a close and detailed observation of how the natural world responds to human environmental changes. As shown in the previous chapter, there can be impacts of forest fragmentation that are not immediately recognizable, and my genetic analysis seeks to continue the theme of thorough evaluation.

Although *Alouatta* spp. can readily adapt to different habitat types including fragments (Crockett & Eisenberg, 1987), there have been studies indicating *Alouatta* preference for very specific forest structures (Wallace et al., 1998). Results from my study on LSBRS Large Forest vegetation structure in relationship to the forest edge indicate that the edge in the Large Forest fragment has trees with a smaller mean DBH and sparser canopy cover than trees in the interior. Refer to previous chapter for a more detailed explanation of how vegetation characteristics influence *A. palliata* habitat quality.

Considering the vegetation edge effects at LSBRS, it is possible that the dispersal behavior of *A. palliata* is subject to the influence of anthropogenic edge formation. *A. palliata* is distinct among non-human primates due to the species' liberal dispersal patterns (Glander, 1992; Clarke & Glander, 2008). Dispersal can be understood as emigration from the birth group and immigration into a different reproductive group, which occurs for a variety of reasons including competition for resources, reduction of competition between related individuals and reduction of inbreeding (Glander, 1992). Up to 79% of male and 96% of female *A. palliata* disperse from birth groups and join other groups within the first 1-4 years of life (Glander, 1980; Clarke & Glander, 1984; Glander, 1992). Glander (1980) notes that the resultant emigration and immigration patterns can lead to a high rate of genetic exchange that can cause neighboring groups to become related over time, lowering the genetic variability among groups—meaning the genetic pools among different groups are largely similar. When high-quality resources are limited, the lower-ranking juveniles disperse so that competition with kin decreases; furthermore, it is possible that competition for food is a better predictor for *A. palliata* dispersal patterns than inbreeding avoidance (Glander, 1992). Mandujano et al. (2004) report that *A. palliata* dispersal is more likely when fragments are characterized by high habitat connectivity, where movement between trees without going to the ground is possible. Thus, habitat characteristics such as food quality and canopy connectivity could influence *A. palliata* dispersal.

Gene flow is the movement of genetic material from one group to another. In *A. palliata*, this is apparent in the dispersal of individuals to new reproductive groups. Van

Belle et al. (2012) report significant genetic differentiation among *Alouatta pigra* social groups occupying the same continuous forest in the absence of any obvious barriers to gene flow. Van Belle et al. (2012) hypothesize that this differentiation is likely due to altered dispersal patterns wherein individuals remain in groups with adult kin, decreasing gene flow among groups and increasing differentiation. Pope (1992) reports high genetic variation among groups of *Alouatta seniculus* belonging to the same local population, possibly linked to males dispersing together resulting in large transfer of genetic material. Altered *Alouatta* spp. dispersal patterns, thus, have been shown to influence the genetic differentiation of groups within populations.

To examine the influence of environmental quality on dispersal behavior, I calculate heterozygosity for 2 microsatellite loci as explained later for interior and edge *A. palliata* subpopulations and the entire population at LSBRS. Heterozygosity is an indicator of genetic variability; heterozygous organisms have two different alleles at a haploid locus. In addition to heterozygosity, I consider indicators of gene flow between the edge and interior subpopulations. Although difficult to quantify, gene flow can be inferred with fixation indices, which measure the amount of genetic variability within populations (Whitlock & McCauley, 1999). Wright's (1965) F -statistics outline the ways in which individuals, subpopulations, and the total population are related in terms of inbreeding. F_{IS} is the measurement of how inbred individuals are within a subpopulation (Weir, 2012). If individuals within the edge and interior subpopulations mate at random, the value F_{IS} for each subpopulation will be 0. If inbreeding occurs within the subpopulation, F_{IS} is greater than 0. F_{IT} measures the inbreeding of individuals relative to the total

population. If F_{IT} is greater than 0, this indicates individuals in the entire population, regardless of subpopulation, are inbred. Finally, F_{ST} measures the genetic differentiation among subpopulations. If *A. palliata* individuals disperse and breed randomly between the edge and interior subpopulations, F_{ST} will be close to 0. An F_{ST} value greater than 0 indicates some factors influence the dispersal and differentiation of the subpopulations away from random mating (Weir, 2012). Hardy-Weinberg equilibrium states that allele frequencies will remain constant from generation to generation given the absence of other evolutionary pressures. Hardy-Weinberg equilibrium and F -statistics will give me the statistical information in order to characterize the genetic structure of the *A. palliata* population.

The main reason for heterozygosity differences among *Alouatta* groups in the same population (in continuous forest) is immigration of individuals from genetically different groups, bringing new genes to the established group (Pope, 1998). *Alouatta* populations occupying forests with limited territory, such as fragments, undergo a more subtle genetic exchange. Males tend to transfer to established groups, maximizing heterozygosity within the group and ultimately decreasing the variance among groups (Pope, 1998). However, this assumes that dispersal is equally likely among groups. *Alouatta caraya* inhabiting fragmented landscapes have been reported to show genetic differentiation among groups, likely due to altered dispersal patterns due to fragmentation (Oklander et al., 2010). My investigation observes if dispersal patterns favor high quality habitat, resulting in an unequal distribution of heterozygosity between edge and interior subpopulations and leading to their genetic differentiation.

Beyond indicating whether or not dispersal of *A. palliata* relates to vegetation edge effects, genetic variability indicates species health, as genetic diversity is one important factor in evolutionary processes. Populations subjected to habitat destruction may be threatened by inbreeding due to bottleneck effects and reduced gene flow, compromising their survival (Wright, 1977). High levels of inbreeding can be defined as inbreeding depression, or decreased biological fitness in a population due to low amounts of genetic diversity. More specifically, if the population of *A. palliata* is comprised of few social groups with similar genotypes, inbreeding is likely to be evident (Milton et al., 2009). Maintaining the genetic stability of a species is a topic of concern for preserving the diversity and range of life on earth (Woodruff, 1989). Often, populations at risk of extinction due to habitat destruction and other factors go extinct before genetic diversity is aptly quantified (Oates et al., 2000; Milton et al., 2009).

Polymorphic microsatellite loci, short tandem repeats of two to six nucleotides in an organism's genome, are an easily accessible and reliable source of genetic variability (Bruford & Wayne, 1993). Microsatellite length varies between individuals due to different numbers of nucleotide repeats (Ellegren, 1992), making this measurement a good indicator of genetic inheritance patterns and heterozygosity. Microsatellite analysis has been used to determine heterozygosity and among-group genetic differentiation of *A. palliata* (Milton et al., 2009), as well as to infer kinship and dispersal patterns of other *Alouatta* species (Oklander et al., 2010; Van Belle et al., 2012). An array of polymorphic microsatellite loci has been published for *A. palliata* (Ellsworth & Hoelzer, 1998; Cortes-Ortiz et al., 2009). Microsatellites do not generally encode genes, and these neutral loci

are useful when estimating F -statistics for population structure (Oklander et al., 2010). At these defined loci, several allele sizes with varying numbers of base repeats are possible. The two loci I used were Ab06 and Apm01; alleles amplified using the PCR technique with published primers are between 265 – 293 base pairs at the Ab06 locus (Goncalves et al., 2004) and between 198–227 base pairs at the Apm01 locus (Van Belle et al., 2012).

My goal is to determine the impact of fragmentation, i.e., the creation of a forest edge, on the genetic diversity of the *A. palliata* population inhabiting the Large Forest at LSBRS. First, I hypothesize 1) if *A. palliata* individuals preferentially disperse from natal groups in edge habitat and join groups in the interior then heterozygosity within the interior subpopulation will be greater than heterozygosity within the edge subpopulation and there will be a significant genetic differentiation between edge and interior subpopulations. Second, I hypothesize 2) that the overall genetic structure of the entire population of *A. palliata* will indicate a heterozygote deficiency due to inbreeding depression. Due to my prediction that the overall population will be deficient in heterozygous individuals, I hypothesize that 3) the population will not be at Hardy-Weinberg equilibrium.

Methods

Study Site

I conducted my research at the La Suerte Biological Research Station (LSBRS) in northeastern Costa Rica. I collected data in the Large Forest fragment. The Methods section in Chapter IV contains a detailed description of the study site.

Data Collection

I used fecal samples as the source of DNA from individuals of the *A. palliata* population at LSBRS. DNA extraction from fecal samples offers a reliable source of host DNA with minimum invasiveness when compared to procedures such as blood sample extraction (Morin & Woodruff, 1996). In the field, I located individual monkeys by listening for their calls and visually identifying them in trees and waited for them to defecate. I collected fecal samples using wooden spatulas and placed samples in 5 mL collection vials containing 2.5 mL of RNA*later* (Thermo Fisher Scientific). I also identified the individual's age class (juvenile or adult) and sex when possible. I recorded existing trail markers as reference points for the location of collection, and a GPS unit was used to mark a waypoint at the exact location, which I recorded on the vial in addition to time of day, species, sex and age class. I stored samples at room temperature until shipment (FedEx) from LSBRS to Regis University for further analysis. Nate Pryor, our class research assistant, uploaded waypoints onto Garmin BaseCamp and collected GPS mapping data for the perimeter of the Large Forest fragment. From these data, I classified fecal collection waypoints as edge or interior samples based on their distance from the edge (greater than 50 m are interior, less than 50 m are edge). Distance was determined using BaseCamp and Google Earth.

DNA Purification

Once samples arrived at Regis University, I sorted, labeled, and stored them at -20° C. I extracted host DNA from fecal samples using the QIAamp DNA Stool Minikit (Qiagen), as the DNA yield for this method, paired with preservation in RNA*later*, is

reliably high (Nechvatal et al., 2008). I followed the protocol that came with the kit with the modification of lysing 1 g of stool in 5 mL of InhibitEX buffer instead of the recommended 10 mL in order to increase the concentration of DNA in downstream steps. I stored purified DNA at -20° C for later PCR analysis.

PCR Amplification and Sequencing

I used the Polymerase Chain Reaction (PCR) technique to amplify polymorphic microsatellite loci in the isolated DNA samples with Apm01 primers (Cortes-Ortiz et al., 2009) and Ab06 primers (Goncalves et al., 2004). I used the following PCR conditions: initial denaturation step of 94 °C for 2 min, followed by 35 cycles of 94 °C denaturation for 20 s, a variable annealing temperature for 20 s, and 72 °C elongation for 45 s, then a final elongation step of 72 °C for 10 min (Nechvatal et al., 2008). The annealing temperatures were 50 °C for Ab06 and 64 °C for Apm01 (Cortes-Ortiz et al., 2009; Goncalves et al., 2004). PCR reactions contained Taq polymerase buffer (New England BioLabs) at a working concentration of 1X, MgCl₂ (Thermo Fisher Scientific) at a concentration of 1.5 mM, dNTPs at a concentration of 200 µM, forward and reverse primers at concentrations of 0.5 µM, and Taq polymerase at a concentration of 1.5 units per reaction. I added approximately 50 ng of DNA to each reaction, and brought the final volume to 20 µL using sterilized water. Concentration of DNA was quantified using a BioMate 3S spectrophotometer (Thermo Scientific).

Once I completed the PCR for a sample, I electrophoresed it on a 1.75% agarose gel at 100 volts. I imaged gels using Bullseye DNA SafeStain (MIDSCI) and an ultraviolet lamp. Upon verification of the presence of alleles within the expected size ranges, I

duplicated PCR for the sample. I then sent 20 μL of the unaltered PCR products, along with 10 μL of the accompanying forward primer, for Sanger sequencing using Big Dye V3.1 and run on ABI 3730xl instruments, completed by Functional Biosciences (Madison, WI). I received sequencing results from Functional Biosciences and read the electropherograms they supplied using 4Peaks (Nucleobytes).

Allele Typing

Due to the low-resolution of gel electrophoresis, it was not possible to discern the presence of separate allele sizes with gel electrophoresis alone. Because sequence data contained a mixture of the two alleles, I was able to analyze electropherograms of each sample to score allele sizes and determine heterozygosity or homozygosity. I constructed a list of rules for the counting of nucleotides in the electropherogram (adapted from *DNA Sequencing Core*, 2016).

1. I started base counting with the first clear occurrence of the repeat of the motif on electropherogram.
2. I continued to count the bases that clearly followed the repeat motif, consisting of tall, distinct base peaks.
3. The last distinct base of the repeat motif marked the end of the length of the first allele.
4. Next, I counted all motif repeats after step 3 if these base pairs were approximately half as tall as the preceding repeats and overlapped by different bases (not the motif), equally as tall.

5. I continued to count the half-height base peaks that clearly followed the repeat motif.
6. The last distinct half-height base peak of the repeat motif marked the end of the length of the second allele, consisting of all bases between steps 1 and 6.

To assess electropherogram quality, I checked the signal intensity for the guanine base peaks, which is a good indicator of the reliability of the electropherogram (DNA Sequencing Core, 2016). I set a threshold signal intensity of 100.

Data Analysis

I evaluated the data by first determining allele frequencies. From this information, I derived all subsequent analyses. I determined Hardy-Weinberg equilibrium for the edge and interior subpopulations and the entire population using GENEPOP (Raymond & Rousset, 1995; Rousset, 2008). This online program tests how well allele frequencies fit the null hypothesis that mating is random and observed heterozygosity and expected heterozygosity values are equal. I tested each subpopulation for adherence to Hardy-Weinberg equilibrium and I tested the entire population for heterozygote deficiency. Results were significant when $p < 0.05$. I estimated Wright's F -statistics, F_{ST} , F_{IT} , and F_{IS} (Wright, 1965) using measurements of heterozygosity and formulae from Peakall and Smouse (2009) (See Boxes 1 and 2).

Box 1: Heterozygosity measurements. Edge and interior are the two subpopulations considered in this study (Peakall & Smouse, 2009).

H_o is observed heterozygosity of the subpopulation and is given as the proportion of N samples that are heterozygous at the locus.

$$H_o = \frac{\text{No. of Heterozygotes}}{N}$$

H_e is expected heterozygosity of the sub-population and is the sum of the squares of allele frequencies subtracted from 1.

$$H_e = 1 - \sum p_i^2$$

\bar{H}_o is mean observed heterozygosity and is the sum of both subpopulation's observed heterozygosity, divided by total number of subpopulations, k .

$$\bar{H}_o = \sum_{i=1}^k H_o / k$$

\bar{H}_e is mean expected heterozygosity and is the sum of both subpopulation's expected heterozygosity, divided by the total number of sub-populations, k .

$$\bar{H}_e = \sum_{i=1}^k H_e / k$$

H_T is total expected heterozygosity and is the sum of the squares of allele frequencies for the entire population subtracted from one.

$$H_T = 1 - \sum_{i=1}^h p_{Ti}^2$$

Box 2: Fixation indices, values for F_{IS} and F_{IT} range from -1 to +1. Values close to zero indicate non-random mating, positive values indicate inbreeding and excess of homozygosity while negative values indicate excess heterozygosity. Values for F_{ST} range from 0 to +1 and greater positive values indicate differentiation among subpopulations (Peakall & Smouse, 2009).

F_{IS} is the inbreeding coefficient within individuals relative to the subpopulation, measuring the reduction in heterozygosity of an individual due to non-random mating within its subpopulation.

$$F_{IS} = \frac{\bar{H}_e - \bar{H}_o}{\bar{H}_e}$$

F_{IT} is the inbreeding coefficient within individuals relative to the total population.

$$F_{IT} = \frac{H_T - \bar{H}_o}{H_T}$$

F_{ST} is the inbreeding coefficient within subpopulations relative to the totalpopulation. This statistic measures the genetic differentiation between subpopulations. F_{ST} values less than 0.05 were interpreted to indicate no evidence of genetic differentiation between subpopulations

$$F_{ST} = \frac{H_T - \bar{H}_e}{H_T}$$

Results

A total of 96 fecal samples were collected. Of these 96, 31 were confirmed from GPS data as monkeys in the edge subpopulation and 26 were identified in the forest interior subpopulation (Figure 8); these samples were used for the genetic analysis. For each subpopulation, I analyzed 20 samples at both the Ab06 and Apm01 loci.

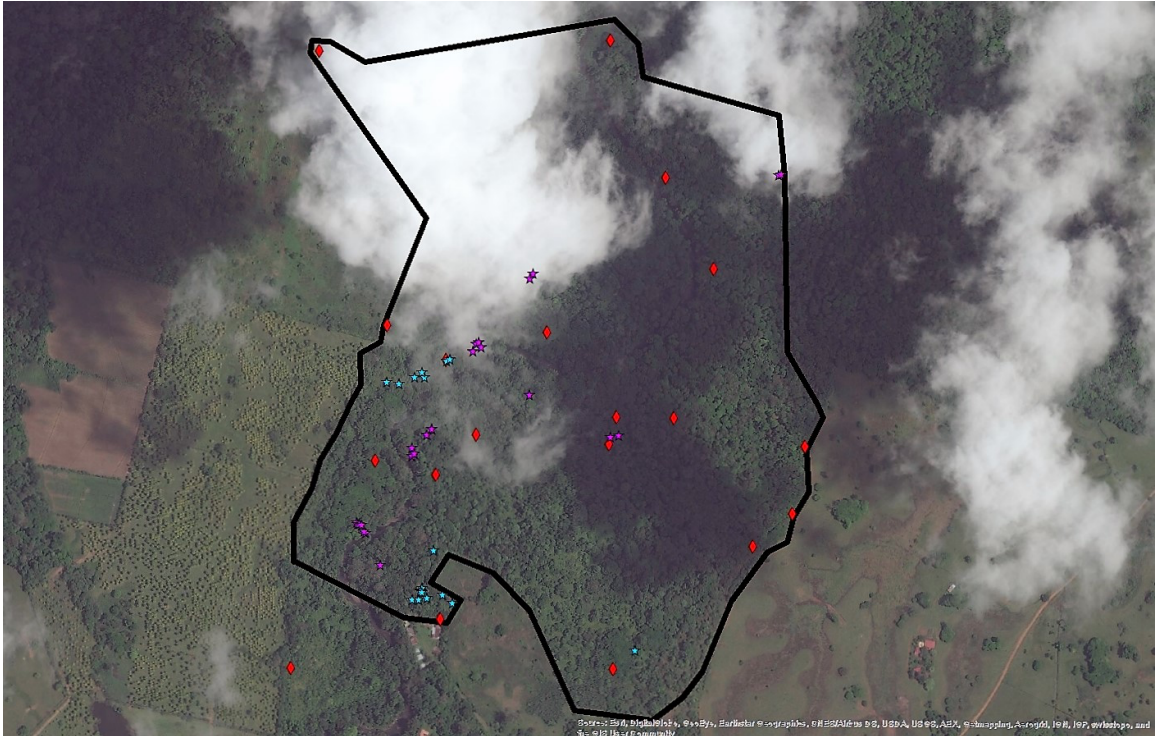


Figure 8: GPS locations of fecal sample collections. Magenta markers indicate interior samples and blue markers indicate edge samples (Map from Nate Pryor).

Gel electrophoresis confirmed the presence of microsatellite alleles in all sequenced samples. Alleles for Apm01 were within the range of 180-208 bp (Figure 9A) and alleles for Ab06 were within the range of 270-280 bp (Figure 9B). Gel electrophoresis did not indicate the presence of any heterozygotes among the individuals sampled; all bands were singular and appeared homozygous. After typing alleles, I found a total of 6 alleles for the Apm01 locus ranging from 31 to 50 bp long, and 6 alleles for the Ab06 locus ranging from 40 to 75 bp long.

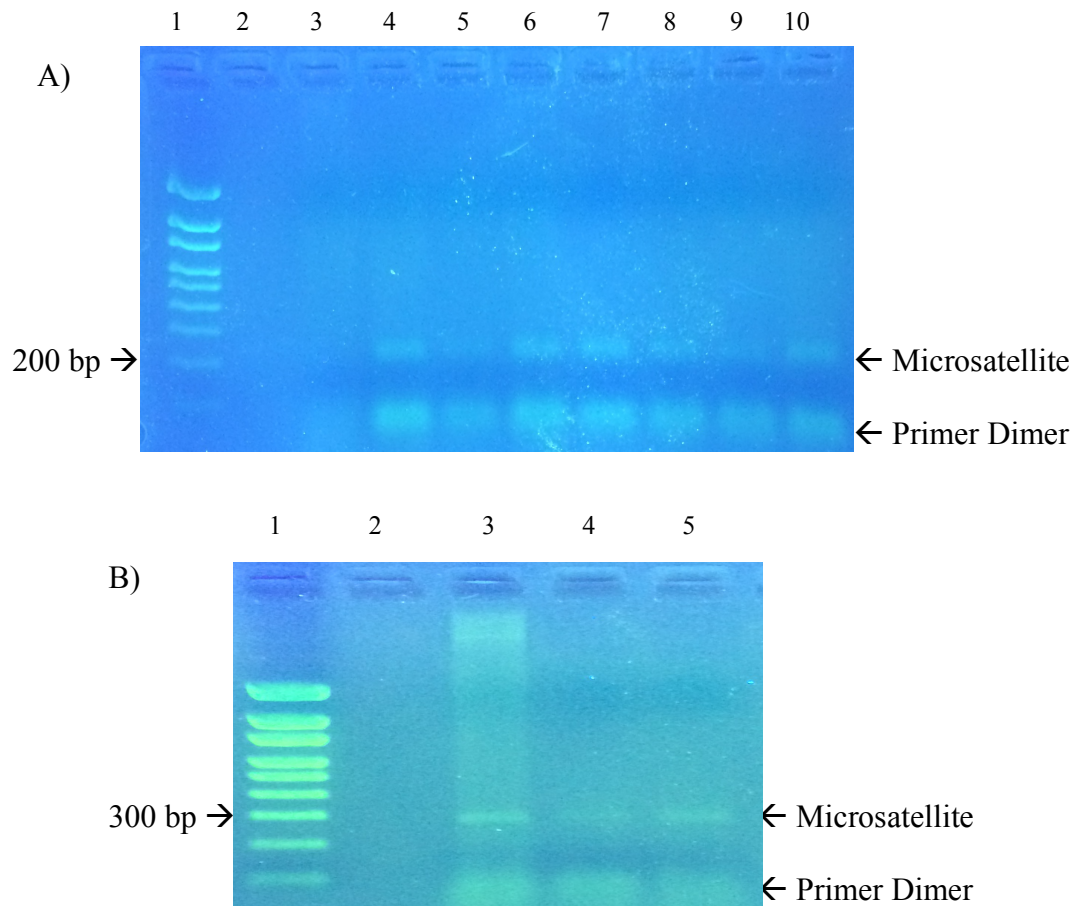


Figure 8: Examples of gel electrophoresis results for both microsatellite loci. A) Amplification of 8 *A. palliata* samples (lanes 3-10) at the Apm01 locus. Microsatellite bands are at approximately 200 bp. The primer dimers, caused by primers annealing together, are the bands at approximately 50 bp. B) Amplification of 3 *A. palliata* samples (lanes 3-5) at the Ab06 locus. Microsatellite bands are at approximately 270 bp. The smudged bands below each lane at 100 bp are primer dimers, resulting from annealing of primer sets together.

All electropherograms had guanine signal intensities above 100 except for 3 samples for the Ab06 locus. However, these samples were still included in the analysis as the peaks were clean and distinct.

I calculated heterozygosity values for each locus and averaged the values over both loci. I report an observed heterozygosity (H_o) of 0.38 for the edge subpopulation and 0.18 for the interior subpopulation. The expected heterozygosity (H_e) was 0.47 and 0.50 for

the edge and interior subpopulation, respectively. Mean observed heterozygosity (\bar{H}_o), averaged between both subpopulations, is 0.28 and mean expected heterozygosity (\bar{H}_e), is 0.49 (Table 1).

Table 1: Observed and expected heterozygosity for edge and interior subpopulations

Subpopulation	Heterozygosity			
	Ho	He	\bar{H}_o	\bar{H}_e
Edge	0.38	0.47	-	-
Interior	0.18	0.50	-	-
Total	-	-	0.28	0.49

Averaged over both polymorphic loci, I calculated hierarchical inbreeding coefficients. The inbreeding coefficient for individuals relative to the subpopulation, F_{IS} , is 0.22 for the edge and 0.65 for the interior subpopulation. The inbreeding coefficient within all individuals relative to the total, F_{IT} , is 0.45. The inbreeding coefficient within subpopulations relative to the total population, F_{ST} , is 0.022 (Table 2).

Table 2: Hierarchical inbreeding coefficients for edge, interior, and total populations

Subpopulation	Coefficient		
	F_{IS}	F_{IT}	F_{ST}
Edge	0.22	-	-
Interior	0.65	-	-
Total	-	0.45	0.022

Allele frequencies in each subpopulation and at each locus are not at Hardy-Weinberg equilibrium ($p < 0.05$). Furthermore, the total population is not at equilibrium ($p < 0.05$), and the Hardy-Weinberg test for heterozygote deficiency among the total population yielded statistically significant results ($p < 0.05$).

In summary, the edge subpopulation has a greater observed and expected heterozygosity than the interior subpopulation. Observed heterozygosity is lower than

expected heterozygosity for both subpopulations as well as the total population. Both subpopulations had positive inbreeding coefficients. Within individuals, with respect to the subpopulations as well as the total, I report positive inbreeding coefficients. Finally, the genetic differentiation between subpopulations, F_{ST} , is below the 0.05 threshold of significance.

Discussion

My first hypothesis, which stated the *A. palliata* subpopulation at LSBRS sampled within 50 m of the forest edge would exhibit lower overall genetic diversity than the subpopulation sampled greater than 50 m from the forest edge, is rejected after genetic analysis. I report that the interior population has both a lower degree of inbreeding and a higher level of heterozygosity than the edge population at LSBRS. However, the indicator for genetic differentiation, F_{ST} , between subpopulations indicates that this difference is not significant.

My second hypothesis, which stated that the entire *A. palliata* population at LSBRS would exhibit signs of inbreeding, is supported by the data presented here. Overall, the population had a lower mean observed heterozygosity than mean expected heterozygosity. Furthermore, individuals indicated a high degree of inbreeding with respect to the expected heterozygosity for both subpopulations and the total population.

Neither subpopulation nor the total population is reported at Hardy-Weinberg equilibrium at both microsatellite loci. I reject the null hypothesis of no evolution and support the alternative hypothesis of evolution. Furthermore, when tested for heterozygote deficiency, Hardy-Weinberg analysis of the total population yields a

statistically significant result, indicating that there is an overall trend toward homozygosity.

The number of alleles reported for the Apm01 locus, 6, is similar to previously published results for *A. palliata*. Cortes-Ortiz et al. (2009) report a total of 8 alleles at this locus in a Mexican howler population. The number of alleles reported for the Ab06 locus, 6, is higher when compared to a total of 5 found in the same Mexican howler population (Cortes-Ortiz et al., 2009). Allele sizes were within the expected size ranges (Cortes-Ortiz et al., 2009). Sequencing confirmed the presence of microsatellite sequences following the expected repeat motifs (Cortes-Ortiz et al., 2009). Overall, the number, size, and repeat motifs of alleles do agree with what has been previously published for *A. palliata* (Cortes-Ortiz et al., 2009).

The expected heterozygosity for both edge and interior subpopulations are comparable to other studies. One *A. caraya* population, living in a fragmented forest in Argentina, is reported to have an expected heterozygosity of 0.50 ± 0.19 , across 11 microsatellite loci (Oklander et al., 2010). This value does agree with the mean expected heterozygosity I report, 0.49. A genetic study of the *A. palliata* population inhabiting the isolated forest fragment, Barro Colorado Island, in Panama is reported to have a slightly greater expected heterozygosity (0.584 ± 0.063) than the population I studied (Milton et al., 2009). Van Belle et al. (2012) report a mean expected heterozygosity of 0.572 for *A. pigra* inhabiting a fragmented forest in Mexico, which is greater than my reported value of 0.49 for the same measurement. Thus, this population of *A. palliata* has a level of genetic variability similar to other *Alouatta* populations inhabiting forest fragments.

Genetic differentiation between the edge and interior subpopulation was negligible. This finding indicates that the edge and interior subpopulation do not have different genetic identities, rejecting my edge effect hypothesis. The reported F_{ST} value of 0.022 agrees with previous studies on *A. palliata* that report F_{ST} values of 0.02 (Ellsworth, 2000; Milton et al., 2009), indicating intergroup movement and liberal dispersal patterns (Van Belle, 2012). Oklander et al. (2010) report significant genetic differentiation between groups of *A. caraya* in a fragment, $F_{ST} = 0.139$. The formation of female matriline potentially causes such differentiation, in which females remain in natal groups, causing groups to remain genetically distinct from one another (Oklander et al., 2010). From the data on genetic differentiation between the two sampled subpopulations, it appears that the population of *A. palliata* at LSBRS has not altered dispersal patterns in response to habitat quality at the edge and interior forest.

The high levels of inbreeding and low levels of observed heterozygosity reported in each subpopulation and in the entire population are interesting results because they indicate the fragmented population has experienced inbreeding. Milton et al. (2009) report an F_{IS} value of -0.067 at the Ab06 locus for a population of *A. palliata* occupying a fragment; though, the analysis over 13 loci gave a group inbreeding coefficient of 0.034 that was not significantly different from 0 (Milton et al., 2009). Oklander et al. (2010) reported negative F_{IS} over multiple loci in *A. caraya* occupying a forest fragment, likely due to inbreeding avoidance. According to F_{IS} measurements, the population of *A. palliata* at LSBRS has experienced inbreeding depression, indicated by an excess of homozygotes.

The observed trend toward allele fixation, or an overrepresentation of one allele, in this population could be a result of random fixation due to genetic drift caused by population size reduction (Keller & Waller, 2002) from deforestation events. It is unclear what type of dispersal patterns might cause the reported values for F_{IS} and F_{ST} . This could be explained by new social group formation, without immigration from genetically different populations (Pope, 1998). Under these conditions, low genetic differentiation would be expected among subpopulations, and heterozygosity would be high due to lack of new genetic material (Pope, 1998). The results I report could also be due to a declining population with random mortality across age and sex classes, fixing alleles by the process of genetic drift, and encouraging the random mating of either males or females among groups (Pope, 1998). Pope (1998) hypothesizes that this situation in *Alouatta* would reduce genetic differentiation among groups and cause decreased heterozygosity relative to Hardy Weinberg equilibrium. My observation that the edge subpopulation has a greater expected heterozygosity, 0.38, when compared to the interior population, 0.18, is potentially explained by immigration of *A. palliata* into the Large Forest fragment via corridors that have been planted at LSBRS. The corridors as well as other trees and patches surrounding this fragment could serve as stepping stones for *A. palliata* immigration (Mandujano et al., 2004), allowing the sharing of genetic material and increasing the observed number of heterozygotes (Pope, 1998) at the edge. Overall, the low heterozygosity of the total population is of potential concern. Populations with reduced genetic diversity are at risk for reduced growth and higher extinction rates (Keller & Waller, 2002).

The primary limitation to the findings presented here is the low number of polymorphic loci sampled, 2, as compared studies that consider at least 11 loci (Cortes-Ortiz et al., 2009; Milton, et al., 2009; Oklander et al., 2010). Allele typing included some uncertainty, as 3 samples were below the sequence quality threshold, however these were still included in the final analysis because I was still able to type them according to the rules I established. My means for estimating howler group home ranges using edge and interior subpopulation delineations is a limited approximation. Studies on the LSBRS primate populations are now being conducted to gather census data on this population, which can be coupled with genetic data in the future.

My results indicate that this *A. palliata* population has experienced a significant degree of inbreeding potentially due to population decline or lack of new immigration events, connected to fragmented forests and deforestation (Milton et al., 2009). However, without a detailed genetic record of the population before deforestation, it is impossible to say for certain. The Maderas Rainforest Conservancy, the non-profit organization that runs LSBRS, has undertaken the creation of corridors to promote increased dispersal between surrounding fragments in hopes of increasing genetic diversity of the different primate populations at LSBRS (Garber et al., 2010). At the end of my time gathering data in the field, my colleagues and I had the opportunity to plant trees and contribute to the growth and expansion of these corridors. Other nation-wide projects include stopping the spread of deforestation along with identifying compromised areas and connecting them with corridors (INBio, 2016). If reforestation continues into the future, I hypothesize that this population of *A. palliata* could interact with surrounding populations, further

diversifying the genetic structure of the population and potentially reversing the observed trend toward inbreeding.

After characterizing the genetic structure of an *A. palliata* population living in a human-made fragment, the results point to a degradation of genetic health. Deforestation for the purpose of supplying tropical fruit to foreign consumers can impact non-human species living in the altered environment. My results are important not only for assessing how genetically healthy a group of primates is, but also for informing the choices of humankind. If industrial-scale farming of a tropical fruit really is important to those who consume such goods, it must be recognized that this process also influences non-human life. Furthermore, the effects likely reach beyond my observations on *A. palliata*'s genetic structure to other species in the same habitat. To maintain a connection with the environment, industrialized civilization must consider the repercussions of its land use.

VI. Final Conclusions

I conclude that there are some measurable trends present in the vegetation structure of the Large Forest at LSBRS as well as in the genetic structure of *A. palliata* that live there. Specifically, I report that the trees at the forest edge are smaller and create a sparser canopy cover than trees in the interior, and that herbaceous ground cover is greater at the forest edge than the interior. These results indicate that the interior of LSBRS Large Forest is composed of higher-quality *A. palliata* habitat when compared to the forest edge. After analyzing the *A. palliata* population's genetic structure, I found no discernable relationship between vegetation structure and the level of inbreeding of edge and interior subpopulations. Thus, my hypothesis that low-quality habitat in the forest edge would induce dispersal to the forest interior was not supported by genetic data. I found that the population does exhibit signs of inbreeding, leading to an excess of homozygous genotypes among the total population, potentially due to low immigration into the forest or a population decline within the fragment.

It is not entirely possible to say if these observations are correlated with the process of deforestation, because the data I present are from only one field season. Despite the limited data, I can state that the forest structure of this fragment is similar to other tropical forest fragments and that the howler monkey population occupying the fragment has a low degree of genetic variability. Both of these phenomena are possibly linked to anthropogenic land alteration.

The stand I took in the second chapter, that civilization is moving toward a separation from the environment, was encouraged, in part, by my travel to Costa Rica, an opportunity not available to everyone. However, everyone living within industrialized civilization at least has the chance to evaluate the issues that I raised. Both urban and rural populations of this civilization play a role in the large-scale system of food production and consumption. Although I concede in the second chapter that industrialization may be beneficial to support a large and dense population, what I put forward in the studies on biological characteristics of a forest fragment require an adjustment of this concession. I have eaten many bananas and pineapples throughout my life, but I never knew what sorts of plants bore them or what environments they grew in. One doesn't have to travel to the tropical banana plantations of the world to appreciate the creative self-sufficiency human beings are known for. The search for truth through empirical observation is a useful tool available to all human beings, as I point out in Chapter III. Purchasing products at the supermarket that rely on a human-built network of trade, deforestation, and commerce to get to the shelf tends to distance human subsistence from the environment that makes it possible, in addition to enabling potentially negative impacts on the non-human species of the world.

I also concede that it is difficult for humans born into the industrialized nations to understand that the food that sustains us comes from an actual environment somewhere else. This challenge applies to all individuals, regardless of groups and class, as long as participation in the production process is absent. One of the great spiritual dangers of living entirely within the sphere of urban life without participating in farming is

“supposing that breakfast comes from the grocery” (Leopold, 1949, p. 6). My thesis was inspired by this concern, even before I read Leopold’s, *A Sand County Almanac* (1949) or traveled to Costa Rica. My second chapter defines this danger as the increasing gap between human beings and the understanding of the environment. Yet, taking time to know the natural world, whether it be tending a community garden or paying attention to current scientific research on the state of the environment, can certainly aid in ameliorating this issue.

I end on a combination of my final conclusion, Darwin’s own scientific discoveries, and Aldo Leopold’s philosophy. According to Leopold (1949, p. 109), thanks to Charles Darwin, modern knowledge now includes the fact that we humans “are only fellow-voyagers in the odyssey of evolution...man, while now captain of the adventuring ship, is hardly the sole object of its quest.” Darwin provided theory that all life on Earth is the product of the never-ending adventure of evolution by natural selection. Leopold reminds us of the interrelatedness of all life through the lens of farming the land and providing the needs for survival. I would like to add the clarification that we human beings are only in control of our own ship, not the entire Earth. As a biological species, we are tasked with fully partaking in the voyage, meaning an utter commitment to sustaining life in such a way that it retains a respect for the vessel that makes it all possible.

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